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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/031,410	06/21/2002	Peter Eriksson	59760 (47137)	2145

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EXAMINER	
MCGILLEM, LAURA L	

ART UNIT	PAPER NUMBER
1636	

DATE MAILED: 03/03/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/031,410	ERIKSSON ET AL.	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 February 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,8-29 and 31-33* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,8-29 and 31-33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *2/7/06, 2/24/06* .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

It is noted that the After Final amendment filed 2/7/06 has been entered and will be considered. Claim 1 has been amended, and claims 30 and 34 have been cancelled.

Finality of the previous Office Action is withdrawn in view of the newly discovered reference(s) to Magae et al (Appl. Micro. Biotechol., 1986, Vol. 24, 509-511) and Pui et al) U.S. Patent No. 6,093,557. Rejections based on the newly cited reference(s) follow.

Claims 1-6, 8-29 and 31-33 are under examination.

Oath/Declaration

The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

> The oath or declaration is defective because:
> Non-initialed and/or non-dated alterations have been made to the oath or declaration. See 37 CFR 1.52(c).

Inventor Moscho has made changes to his post office address, which do not appear to be initialed.

Claim Objections

Claim 5 is objected to because of the following informalities: There is a period after the word "electrical". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-6, 8-29 and 31-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for *in vitro* selective electrofusion of at least two fusion partners having cell-like membranes, does not reasonably provide enablement for *in vivo* electrofusion of two fusion partners, or for conducting *in vitro* fertilization by selective electrofusion of an egg cell or an enucleated egg cell, and a sperm cell at any development stage, or for conducting non-human cloning. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the application coupled with information known in the art without undue experimentation *United States v. Telectronics*, Inc., 8 USPQ2d 1217 (Fed. Cir. 1988). Whether undue experimentation is required is not based upon a single factor, but rather is a conclusion reached by weighing many factors. These factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter. 1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

1) Scope of the claims. The claims are drawn to methods of selective electrofusion of at least two fusion partners having cell-like membranes using an electric

field of a strength sufficient to obtain fusion provided by at least one microelectrode that is sufficiently small to permit selective fusion. The claims encompass any combination of at least two fusion partners having cell-like membranes including cells, liposomes, proteoliposomes, synthetic vesicles, egg cells, enucleated egg cells, sperm cells at any development stage and plant protoplasts. The claims encompass *in vitro* fertilization using egg cells, enucleated egg cells, and sperm cells at any development stage from any species. The claims encompass cloning, excluding human cloning, of any other species besides humans. The claims are drawn to *in vivo* selective electrofusion including administration of pharmaceutically active substances to a cell or to a tumor, which encompasses a very large group of undisclosed pharmaceutically active substances and fusion partners as well as a large group of possible *in vivo* cells or tumors. The scope of the claims is broad and far reaching. The specification does not disclose or contemplate the use of egg cells, enucleated egg cells or sperm cells at any development stage for any use other than *in vitro* fertilization or non-human cloning.

2) State of the Art. A recent review by Sakai et al (Birth Defects Res. C Embryo Today 2005., Vol. 75. No. 2. pp.151-62) teaches that several mammalian species have been cloned by somatic cell nuclear transfer, but that the short and long term effects of cloning and assisted reproductive techniques are largely unknown (see page 152, left column, 1st paragraph). Sakai et al teach that systematic studies of cloned offspring are necessary, but are complicated by the low proportion of live offspring from nuclear transfer eggs, which is currently 2-3% regardless of species or nuclear transfer technique (see page 152, center column, 1st full paragraph). Sakai et al

teach that studies of cloning and cloned animals are limited by the ability to generate a sufficient number of age-matched cloned animals and difficulty in designating appropriate controls (see page 152, right column, 1st paragraph). Sakai et al teach that reasons for low efficiency of cloning are currently unclear, but that exposure of eggs and embryos to *in vitro* culture conditions, such as culture medium that may contain chemicals at non-physiological concentrations, can affect embryonic development and result in offspring abnormalities (see page 159, right column, 1st full paragraph, for example). Sakai et al teach that although *in vitro* fertilization techniques are widely used in livestock production, *in vitro* embryo culture has been associated with abnormal physiology and morphological development and a perinatal mortality at a higher rate than natural fertilization. Niemann and Rath (Theriogenology, 2001 Vol. 56. No.8. abstract) teach that there are differences in the progress of *in vitro* reproductive techniques among livestock such as cattle, sheep and swine. Sakai et al suggests that the amount of cellular trauma and damage to eggs, sperm and embryos during manipulation could result in negatively impacted cellular development and may depend on the technical skill of the individual manipulating the gametes and embryos (see page 160, left column, 1st paragraph, for example).

Orentas et al (Cell Immunol. 2001., Vol. 213, No. 1, pp 4-13) teach that cellular fusion can be used to generate anti-tumor immunity treatments such as by fusion of dendritic cells containing tumor-derived material with other cell types. Orentas et al disclose that such fusions have previously been performed using polyethylene glycol (PEG) but that electrofusion is superior to PEG-mediated fusion and may be useful to

produce more potent cancer vaccines (see page 4, right column, 3rd paragraph and page 12, left column, 4th paragraph, for example). Mekid and Mir (Biochim Biophys Acta. 2000. Vol. 1524. No. 2-3, pp.118-30) teach that electropulses are used in electrochemotherapy to introduce drugs into tumor cells and DNA electrotransfer for gene therapy. Mekid and Mir found unexpected electrofusion of tumor cells as a result of electropulse methods and variability in fusion among different tumor cell types (see page 129, left column). Mekid and Mir suggest that the potential for electrofusion of tumor cells from electropulses, as well as introduction of cytotoxic drugs by electrochemotherapy or DNA electrotransfer could convey a therapeutic advantage by increasing the chance of cell death even in cells which did not receive lethal doses of therapeutic drugs or genes (see page 129, left column, 1st and 2nd paragraph).

3) Unpredictability of the art. The unpredictability of using methods of *in vitro* fertilization and cloning is manifested in multiple issues. Sakai et al teach that cloning methods and studies of cloning outcomes are hampered by the very low rate of clones produced and the difficulty of establishing appropriate controls. Sakai et al preliminarily conclude that clones are not always phenotypically identical to the somatic cell donors and that the cloned progeny often have adverse health conditions, such as increased body weight and advanced aging. Sakai et al teach that cloning technique is "still unpredictable" and requires comprehensive and systematic longitudinal studies of cloned animals (see page 152, right column, 1st paragraph). Sakai et al disclose that pre- and perinatal death rates in clones are significantly higher than controls regardless of species and that the reason for low efficiency of somatic cell cloning are currently

unclear (see page 153, center column, 1st paragraph). Sakai et al teach that the variety of findings in cloned animals of multiple species suggests that cloning has different consequences among different species and even within a species.

Niemann and Rath (Theriogenology, 2001 Vol. 56. No.8. abstract) teach that success rates for *in vitro* fertilization of porcine embryos is much lower than that of cattle. Niemann and Rath teach that main problems for *in vitro* fertilization for swine include insufficient cytoplasmic maturation of oocytes, low proportion of blastocysts and high proportion of polyspermic fertilization (see abstract). Therefore, the unpredictability being able to use the claimed methods of cloning and *in vitro* fertilization is based on art-recognized unpredictability of similar methods even between species and that the specification has not taught how to use the claimed methods to successfully conduct cloning or *in vitro* fertilization.

Mekid and Mir (Biochim Biophys Acta. 2000. Vol. 1524. No. 2-3, pp.118-30) teach variability in results from *in vivo* cell electrofusion. Mekid and Mir treated mouse B16 melanoma and LPB sarcoma tumors and mouse liver with electrical pulses in order to electropermeabilize cells (see page 119, right column, 1st paragraph). Mekid and Mir teach that the effect of the electrofusion was tissue type-dependent. The B16 melanoma tumor cells fused and formed syncytial areas and giant cells, but using the same electropulse method and the same electrode, the LPB sarcoma cells did not produce the same results as the B16 cells, even at higher voltages (see page 124, right column and page 125, left column, for example). Mouse liver was also subjected to the same electropulse as the tumor cells and did not result in electrofusion of the tumor cells,

even after an increase in voltage (page 125, right column, for example). Mekid and Mir suggest that the difference in electrofusion ability between tumor types and normal tissue is related to the presence of proteases in the interstitial fluid which might have reduced the surrounding extracellular matrix to bring the adjacent cells in closer contact (see page 128, right column, last paragraph). One of skill in the art would also recognize that such *in vivo* electrofusion methods (i.e. multiple liposomes containing a pharmaceutical fused with an *in vivo* cell or a network of cells) might result in uneven fusion among adjacent cells wherein one cell may fuse with many liposomes, but an adjacent cell may fuse with only one liposome or none at all. Therefore, the success of the claimed methods drawn to *in vivo* electrofusion and treatment of tumors or introduction of pharmaceuticals is unpredictable, because not all tumor cell types are subject to equal fusion using similar methods.

4) Amount of guidance provided. The specification has provided some guidance regarding the specific number, strength and duration of fusion pulses required to fuse several well-known cell types *in vitro*. The specification has provided some guidance regarding the size of a hollow electrode, including the dimensions of a specific electrode used to fuse two cells *in vitro*. The specification provides broad ranges for the electrical field strength necessary for fusion (i.e. 0.1-10 kV/cm) for pulses of 10 μs to several seconds in duration. The specification discloses that for method using multiple pulses a repetition rate of ~1 Hz should be suitable. The specification discloses that the length and strength of the pulses depend on the size of the partners to be fused. The specification provides preferable size ranges for the microelectrodes

such as an outer diameter of a few nm to ~100 μm, preferably 5-30 μm and most preferably 20 μm. The Applicants have not provided any guidance regarding the specific size and number of electrodes to be used for *in vivo* electrofusion, non-human cloning or *in vitro* fertilization. Applicants have not provided any guidance regarding the specific number, strength and duration of fusion pulses to be used for *in vivo* electrofusion, non-human cloning or *in vitro* fertilization. Applicants have not provided any guidance regarding the electrical field to be used for *in vivo* electrofusion, non-human cloning or *in vitro* fertilization. Applicants have not provided any guidance regarding differences in the claimed fusion method for any other cells beside those exemplified regarding potential variations related to cell type, age and growth conditions, especially for non-human cloning and *in vitro* fertilization. Applicants have not provided any information concerning any variations in the claimed method for various pharmaceutical agents to be delivered to cells or tumors. Therefore the Applicants have not provided guidance to perform the full scope of the claimed method without undue trial and error experimentation.

5) Working examples. Applicants have provided an example of cell-cell fusion of PC12 cells *in vitro* with information regarding specific number, strength and duration of fusion pulses. Applicants disclose but do not show: fusion of NG108 cells in a network, fusion of NG108 cells, Jurkat cells and COS7 cells, and fusion of NG108 cells to PC12 cell to create hybrid cells. Applicants have provided an example of cell-single vesicle fusion in which the cells have been pretreated with a protease, and do not provide information regarding specific number, strength and duration of fusion pulses.

Applicants have provided a third example in which NG108 cells are fused using electrolyte-filled silica capillary electrodes which are defined by their dimensions and include information regarding specific number, strength and duration of fusion pulses required to fuse the cells. Applicants have not provided any example of non-human cloning, *in vitro* fertilization of any organism or electrofusion to deliver pharmaceutically active agents *in vivo* or for tumor treatment.

6) Nature of the invention. The nature of the invention is drawn to in vivo cell electrofusion, *in vitro* fertilization and cloning of non-human organisms, which are very complex and controversial aspects of science and medicine to date.

7) Level of skill in the art. The level of skill in the art is low because the Applicants have not reduced that claimed method to practice.

Given the above analysis of the factors which the Courts have determined are critical in ascertaining whether a claimed invention is enabled, it must be considered that the skilled artisan would have had to have practiced undue and excessive experimentation in order to practice the claimed invention.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the

applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claim 1 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Magae et al (Appl. Micor. Biotechol., 1986, Vol. 24, 509-511). This is a NEW REJECTION.

Magae et al teach a method to fuse two giant plant protoplasts by using two glass electrodes prepared from glass capillaries and attached to a micromanipulator. Two protoplasts were brought into contact with each other and a single electrical pulse was applied to the protoplasts to fuse the membranes (see page 509, right column, 3rd full paragraph) which reads on a method comprising bringing into contact two fusion partners having cell-like membranes and providing an electric field using at least one microelectrode which is of a strength to obtain fusion and highly focused on the fusion partners. The electrodes are positioned by means of a micropositioner and at least one microelectrode is hollow and sufficiently small to permit the selective fusion of two fusion partners. Magae et al teach that the protoplast must be prepared in cellulose so that the protoplasts were large enough for the electrode and were suitable for electrical fusion in 0.6 M mannitol containing $CaCl_2$ (see page 510, left column, 2nd paragraph, for example) which reads on the electrode being sufficiently small to permit selective fusion of the protoplasts and providing the fusion partners in a buffer prior to fusion.

Claims 1- 2, 8-12, 15-19 and 26-29 are rejected under 35 U.S.C. 102(e) as being anticipated by (Pui et al) U.S. Patent No. 6,093,557, filed 6/5/1998. This is a NEW REJECTION.

Pui et al teach a method and apparatus for fusion of cells with vesicles or liposomes that comprises a capillary electrode through which the liposomes are dispersed in a spray on to target cells (see column 11, lines 6-15, column 14, lines 52-64, for example). An electrical charge is created between a high voltage liposome-dispensing capillary tube electrode and a second electrode so that the liposomes are fused with the target cells (see column 15, lines 14-25, for example). The instant specification discloses that, in the case of using a single electrode, the electrode is preferably an anode and works against a grounded cell preparation (see specification page 9, lines 1-4). Pui et al teach that a high positive voltage can be applied to the first electrode and the second electrode is grounded, and also teach that the second electrode can be a grounded ring electrode and a ground target surface holding the cells (see column 7, lines 5-9 and column 9, lines 46-55, in particular), which reads on the method wherein only one microelectrode is used to provide the electrical field. Pui et al teach that the electric field provided between the capillary tube electrode and the grounded electrode provides for the dispensing of the spray (see column 9, lines 35-45 and column 10, line 40, for example) which reads on a method wherein a small, hollow electrolyte-filled microelectrode is used to deliver fusion partners by electrophoresis. Pui et al teach that the spray can be confined to one or more target cells (see column 3, lines 14-20, column 5, lines 33-35, column 6, lines 26-31, column 6, lines 46-65, in

particular), which reads on bringing into contact at least two fusion partners having cell-like membranes and providing an electric field using at least one microelectrode which is of a strength sufficient to obtain fusion and highly focused on the fusion partners wherein at least one microelectrode is hollow (i.e. capillary tube) and sufficiently small to permit selective fusion of two fusion partners. The spray of liposomes also reads on the method wherein one or more of the fusion partners is constituted by a multiple of a structure such as liposome or vesicle. Pui et al teach that the spray dispenser is movable along the x, y, z axes (see column 17, lines 2-5, in particular) which reads on positioning at least one microelectrode by use of a micropositioner and/or stereotactic device. Pui et al disclose that the outer diameter of the capillary tube has a preferred range of 6 μm to about 2.5 mm, or 8 μm to about 2.5 mm (see column 12, lines 55-60, for example), which reads on an electrode with an outer diameter of 1-100 μm. Pui et al teach that the target cells can comprise cells, eggs or plant protoplasts, may be part of a tissue, or a multilayer of cells (column 13, lines 5-24, and column 17, lines 25-30, for example) which reads on the method wherein at least one of the fusion partners is a cell and the other fusion partners are liposomes, a synthetic vesicle, an egg cell, a plant protoplast or is part of a cellular network. Pui et al teach that the liposome or vesicle particles are in a suspension liquid such as a buffer or electrolyte solution (see column 10, lines 37-41, for example), which reads on the claimed method wherein the fusion partners are provided in a buffer prior to fusion. Pui et al teach that the target cells can be a monolayer of cells as well as being affixed to a surface (see column 13, lines 20-25 and column 22, lines 62-64, for example) which reads on the claimed method wherein

at least one of the fusion partners has been immobilized prior to step A. Pui et al teach that liposomes can fuse to the cell membrane (see column 14, lines 52-65, for example), which reads on manipulation of the composition of the cellular membrane since the lipids of the liposome will now be present in the cellular membrane. Pui et al disclose that controlled flow and a known concentration of biological material, the amount of biological material in the spray can be controlled and is reproducible (see column 14, lines 33-40, for example), which reads on the delivery of a well-defined volume of a substance to a cell. Pui et al teach that DNA, RNA, small molecules, bioactive substances and drugs can be delivered in the carrier particles or liposomes (see column 11, lines 6-14, in particular), which reads on a method of delivery of a pharmaceutically active substance to a cell. Pui et al teach that the delivery method can be used to deliver substances to tumor tissue for gene therapy (see column 17, lines 29-33, for example), which reads on the claimed method for the treatment of a tumor.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Laura McGillem, PhD
2/27/2006

DAVID GUZO
PRIMARY EXAMINER